

b4

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percent inhibition

100
80
60
40
20
0

DP178 0.0001 0.001 0.0025
CD4-IgG2 0.0025
or mixture

0.01 0.1 1
0.25 2.5 25

concentration, $\mu\text{g/ml}$

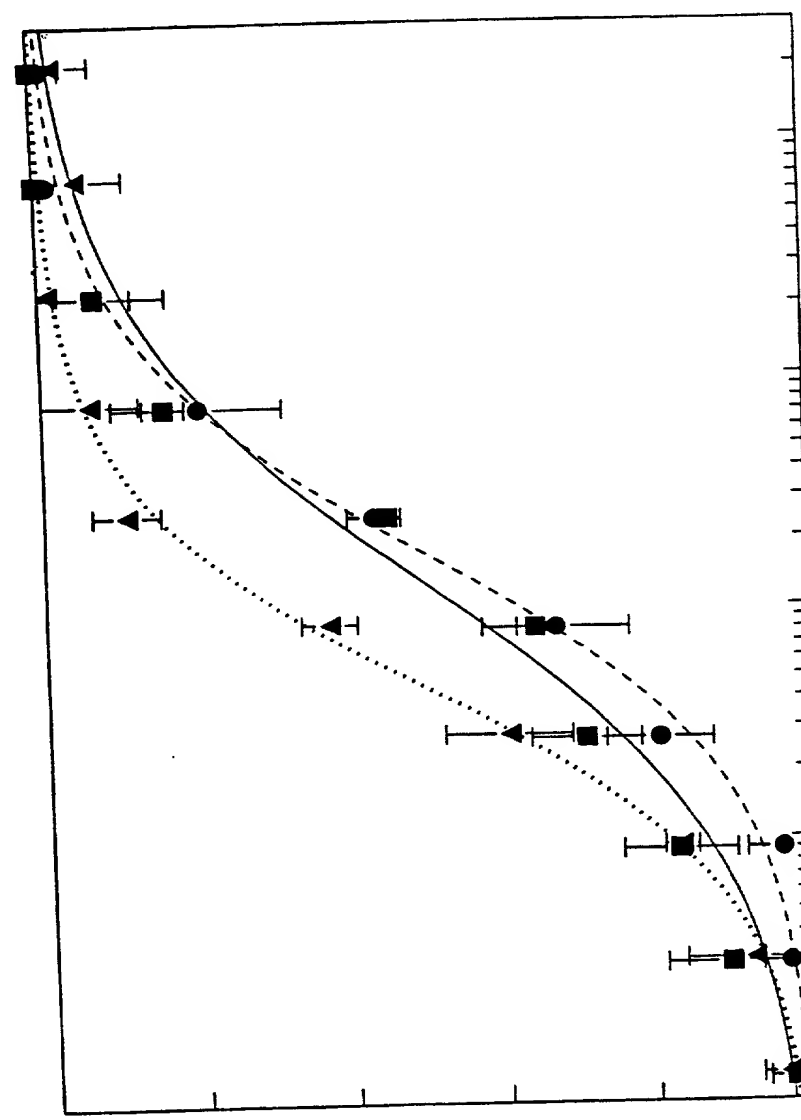


Figure 1

Figure 2

Percent Inhibition	Combination Index			
	CD4-IgG2:T-20 Mass Ratio			
	25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 3

Percent Inhibition	T-20			CD4-IgG2		
	Concentration, µg/ml		Dose Reduction	Concentration, µg/ml		Dose Reduction
	Alone	Combination		Alone	Combination	
99	1.1	0.17	6.6	130	4.3	29
95	0.21	0.044	4.9	19	1.10	17
90	0.10	0.024	4.2	7.8	0.59	13
70	0.025	0.0076	3.3	1.6	0.19	8.4
50	0.011	0.0039	2.8	0.60	0.095	6.3

Figure 4A

Percent Inhibition	Combination Index	PRO 542				PA12				T-20			
		Concentration, nM		Dose		Concentration, nM		Dose		Concentration, nM		Dose	
		Alone	Mix	Reduction	Alone	Alone	Mix	Reduction	Alone	Alone	Mix	Reduction	Alone
95	0.41	10	2.1	4.8	730	2.8	2.8	260	94	19	19	4.9	
90	0.45	7.0	1.6	4.4	320	2.1	2.1	150	63	14	14	4.5	
70	0.47	4.1	0.92	4.5	72	1.2	1.2	60	30	8.1	8.1	3.7	
50	0.48	3.1	0.66	4.7	28	0.87	0.87	32	19	5.8	5.8	3.3	

PRO 542, PA12 and T-20 were used in an approximate 1:1:10 molar concentration ratio.

Figure 4B

Percent Inhibition	Combination Index	PRO 542				PRO 140				T-20			
		Concentration, nM		Dose		Concentration, nM		Dose		Concentration, nM		Dose	
		Alone	Mix	Reduction	Alone	Mix	Reduction	Alone	Mix	Alone	Mix	Reduction	Alone
95	0.40	8.5	1.9	4.5	19	1.0	19	140	17	140	17	8.2	
90	0.39	7.1	1.5	4.7	13	0.77	17	100	13	100	13	7.7	
70	0.37	5.3	0.87	6.1	7.2	0.46	16	57	7.7	57	7.7	7.4	
50	0.35	4.6	0.63	7.3	4.9	0.34	14	40	5.6	40	5.6	7.1	

PRO 542, PRO 140 and T-20 were used in an approximate 2:1:20 molar concentration ratio.

Figure 4C

		PRO 542				PRO 140				T-20			
Percent Inhibition	Combination Index	Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction		Concentration, nM		Dose Reduction	
		Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix	Alone	Mix
95	0.24	61	2.5	24		11.9	0.72	17		156	22		7.1
90	0.22	32	1.4	23		8.4	0.40	21		96	13		7.4
70	0.19	9.8	0.50	20		4.5	0.14	32		40	4.5		8.9
50	0.18	4.7	0.26	18		3.0	0.074	41		23	2.3		10

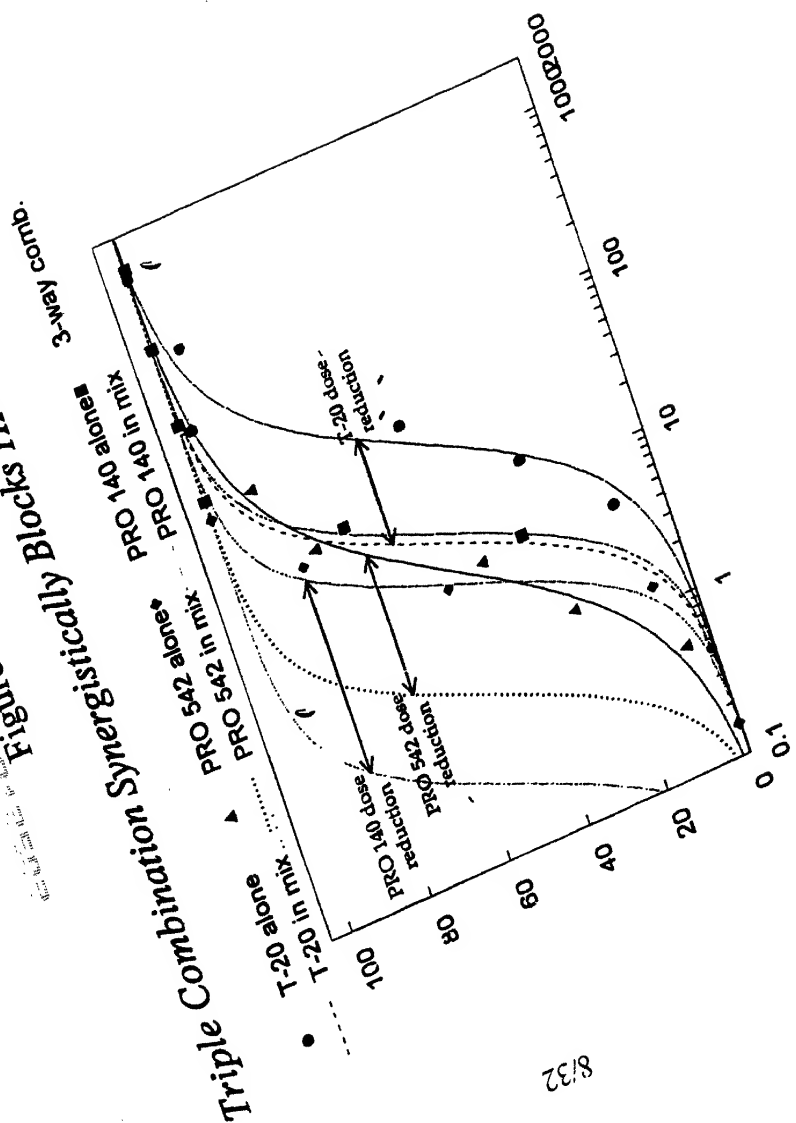
PRO 542, PRO 140 and T-20 were used in an approximate 4:1:30 molar concentration ratio.

Figure 4D

		PRO 140			T-20		
Percent Inhibition	Combination Index	Concentration, nM		Dose Reduction	Concentration, nM		Dose Reduction
		Alone	Mix		Alone	Mix	
95	0.56	12	1.8	6.7	156	55	2.8
90	0.55	8.4	1.1	7.4	96	35	2.7
70	0.55	4.5	0.51	8.8	40	16	2.5
50	0.56	3.0	0.31	9.9	23	10	2.4

PRO 140 and T-20 were used in an approximate 1:30 molar concentration ratio.

Figure 5
Triple Combination Synergistically Blocks HIV-1 Entry (1)



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Figure 6

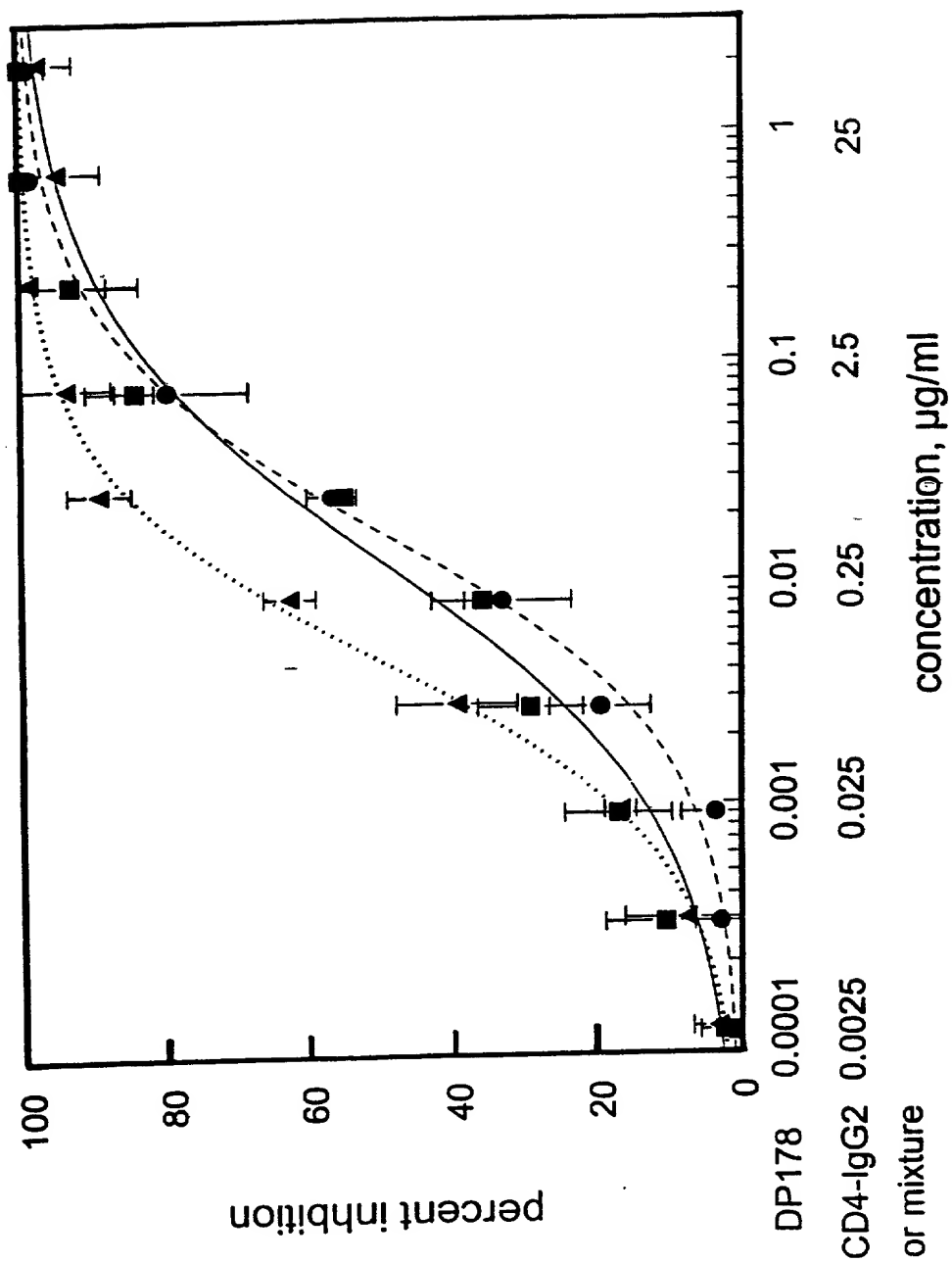


Figure 7

Combination Index				
CD4-IgG2:T-20 Mass Ratio				
Percent Inhibition	25:1 (low)	25:1 (high)	5:1	1:1
95	0.32	0.20	0.22	0.50
90	0.38	0.25	0.27	0.55
85	0.43	0.29	0.30	0.59
80	0.47	0.33	0.34	0.62
75	0.51	0.36	0.37	0.65
70	0.54	0.39	0.40	0.67
65	0.58	0.42	0.43	0.70
60	0.61	0.45	0.45	0.73
55	0.65	0.48	0.49	0.75
50	0.69	0.51	0.52	0.78

Figure 8

Percent Inhibition	T-20			CD4-IgG2		
	Concentration, $\mu\text{g/ml}$		Dose Reduction	Concentration, $\mu\text{g/ml}$		Dose Reduction
	Alone	Combination		Alone	Combination	
99	1.1	0.17	6.6	130	4.3	29
95	0.21	0.044	4.9	19	1.10	17
90	0.10	0.024	4.2	7.8	0.59	13
70	0.025	0.0076	3.3	1.6	0.19	8.4
50	0.011	0.0039	2.8	0.60	0.095	6.3

Figure 9

Assay (virus)	PRO 542:T-20 Molar Ratio	Percent Inhibition	Combination Index	PRO 542		T-20	
				Concentration, nM		Concentration, nM	
				Alone	Mix	Alone	Mix
Virus-cell fusion (JR-FL)	1:2	95	0.14	30	2.8	120	5.1
		90	0.18	12	1.5	45	2.6
		70	0.29	2.5	0.44	8.0	0.78
		50	0.39	0.92	0.21	2.7	0.37
Virus-cell fusion (DH123)	1:2	95	0.36	65	11	123	20
		90	0.45	20	5.0	54	8.9
		70	0.76	2.4	1.2	12	2.1
		50	1.1	0.64	0.49	4.8	0.87
Cell-cell fusion (JR-FL)	1:2	95	0.36	35	6.3	73	11
		90	0.43	14	3.2	34	5.8
		70	0.61	2.9	0.94	8.5	1.7
		50	0.76	1.0	0.43	3.6	0.78
Cell-cell fusion (JR-FL)	1:10	95	0.27	28	1.4	58	12
		90	0.28	11	0.55	22	4.9
		70	0.31	2.3	0.11	3.8	0.97
		50	0.34	0.84	0.039	1.3	0.35
Cell-cell fusion (JR-FL)	1:50	95	0.33	47	0.84	120	37
		90	0.34	15	0.30	42	13
		70	0.36	1.8	0.045	6.1	2.0
		50	0.38	0.49	0.014	1.8	0.61

Figure 10

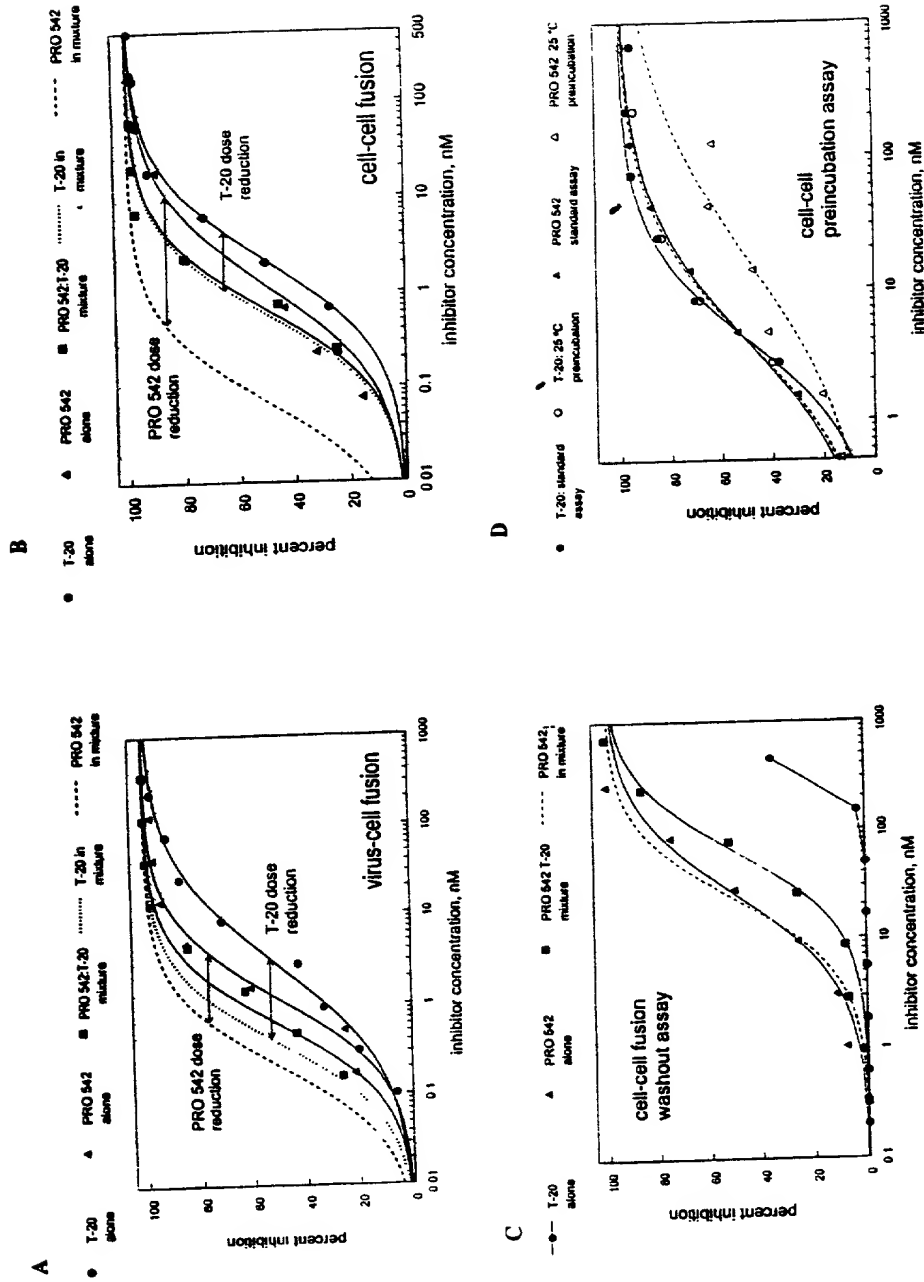


Figure 11

HIV-1 Entry Involves at Least Three Steps that Provide Promising Targets for Therapy

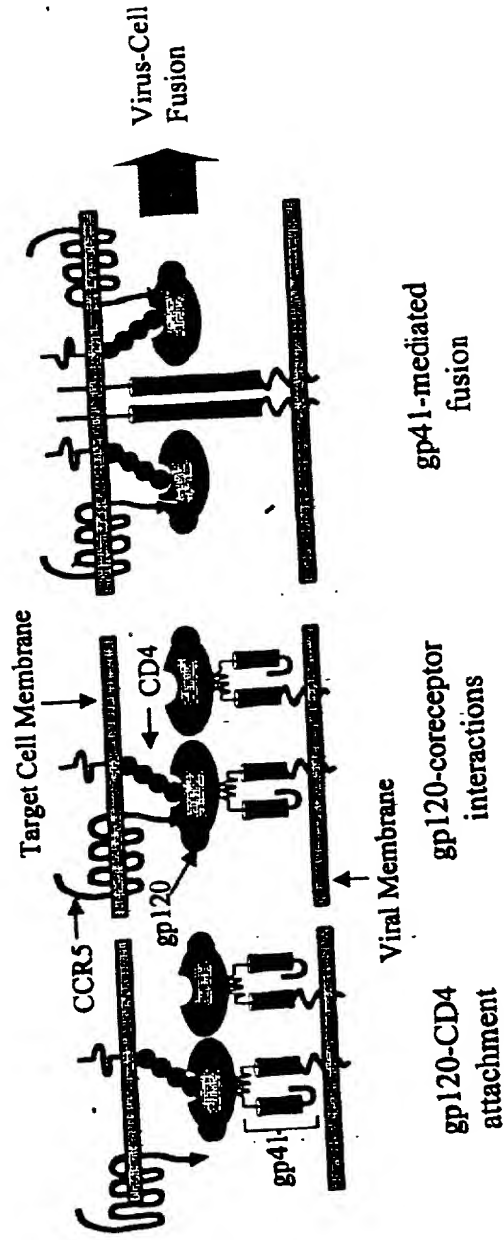


Figure 12

PRO 542 (CD4-IgG2)
attachment inhibitor

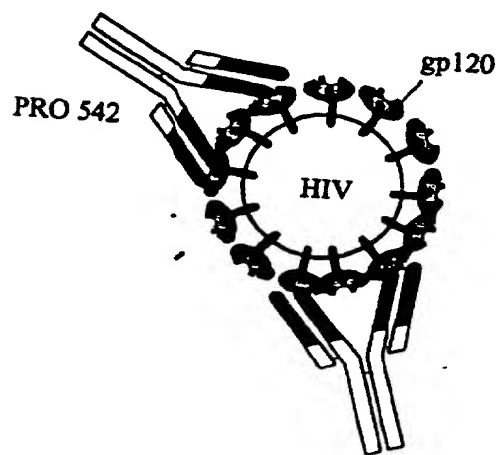


Figure 13

PRO 140
coreceptor inhibitor

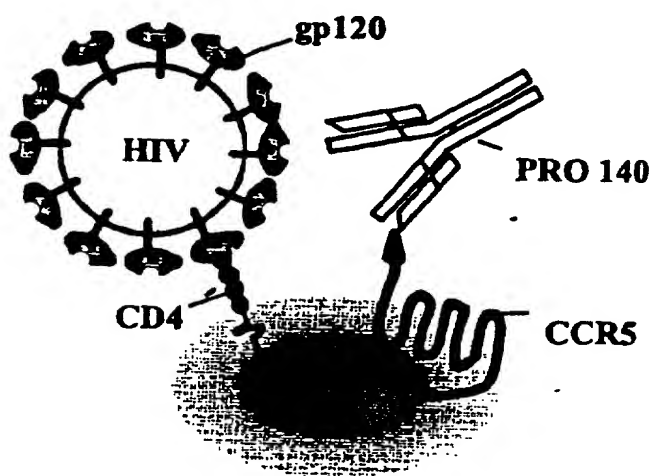


Figure 14

T-20
fusion inhibitor

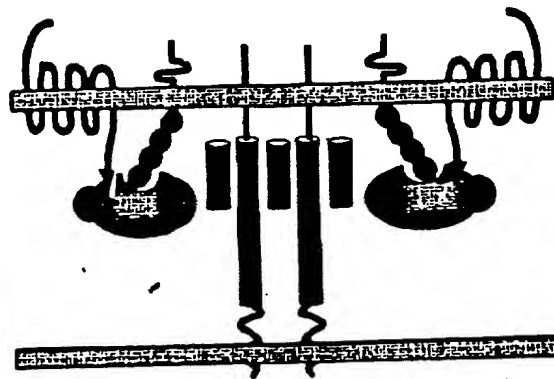


Figure 15

HIV-1 Virus-Cell Fusion Assay

Env⁺ Luc⁺ HIV

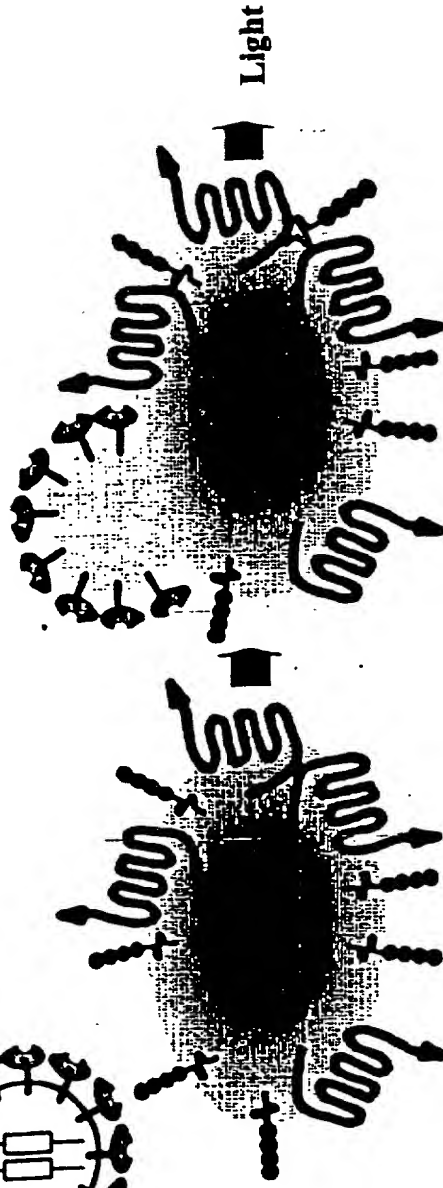
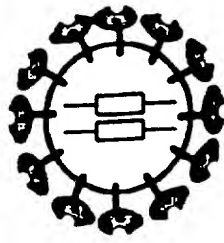


Figure 16

Synergistic Inhibition of Virus-Cell Fusion with PRO 542 and T-20 (I)

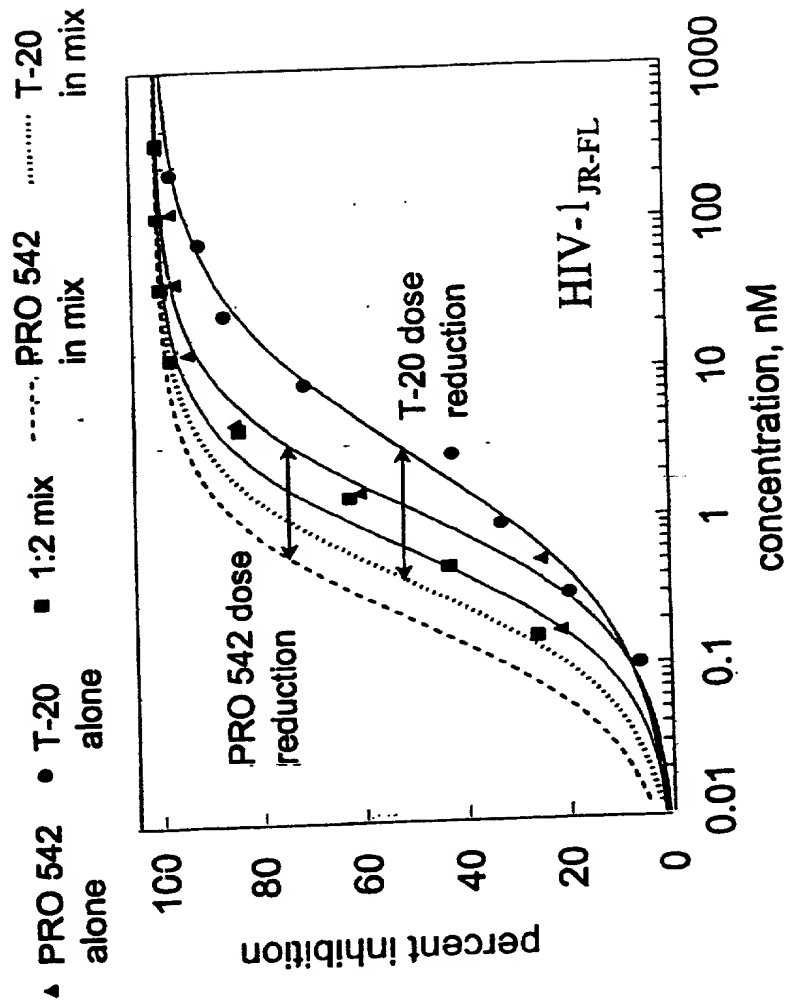


Figure 17

***Synergistic Inhibition of HIV-1 Virus-Cell-Fusion
with PRO 542 and T-20 (II)***

Percent Inhibition	Combination Index	Inhibitory Conc., nM		Dose Reduction	
		PRO 542	T-20	PRO 542	T-20
JR-FL 95	0.14	30	120	11	24
(R5) 90	0.18	12	45	8.0	17
70	0.29	2.5	8.0	5.7	10
50	0.39	0.92	2.7	4.4	7.3
DH123 95	0.36	65	123	5.9	6.2
(R5X4) 90	0.45	20	54	4.0	6.1
70	0.76	2.4	12	2.0	5.7
50	1.1	0.64	4.8	1.3	5.5

PRO 542 and T-20 were used in a 1:2 molar ratio

Figure 18

HIV-1 Cell-Cell Fusion Assay

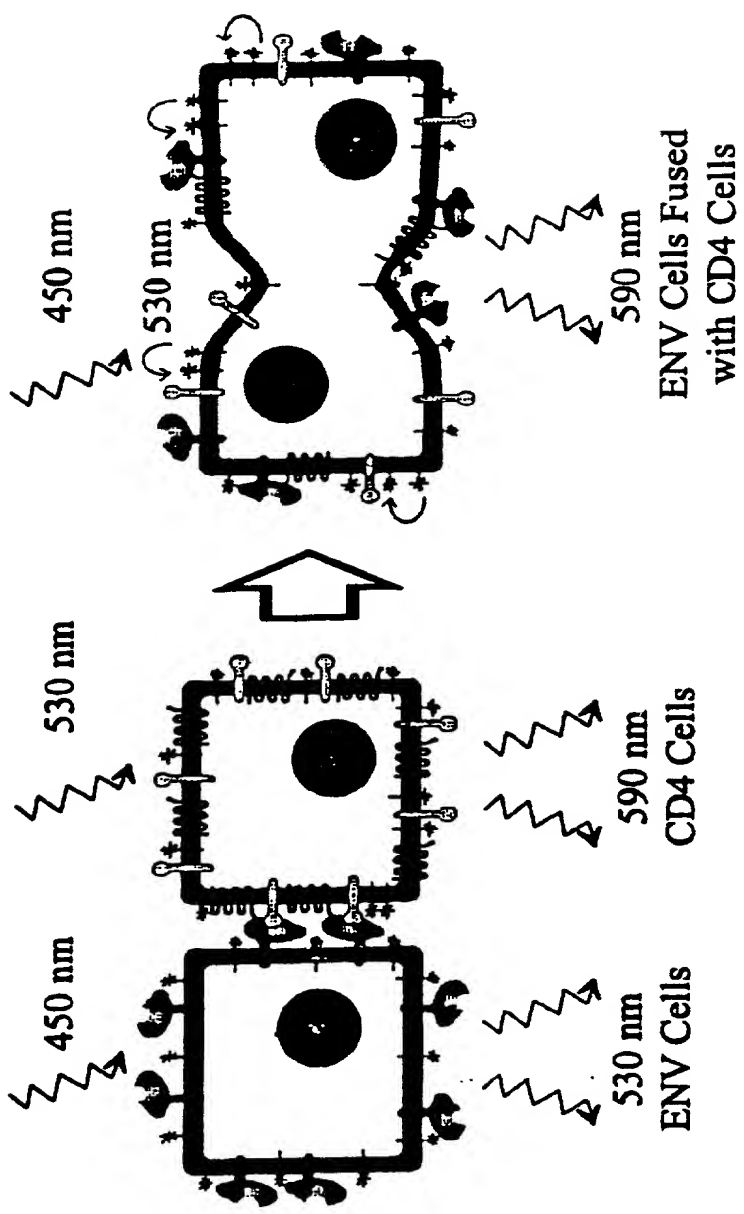


Figure 19
Synergistic Inhibition of Cell-Cell Fusion
with PRO 542 and T-20 (I)

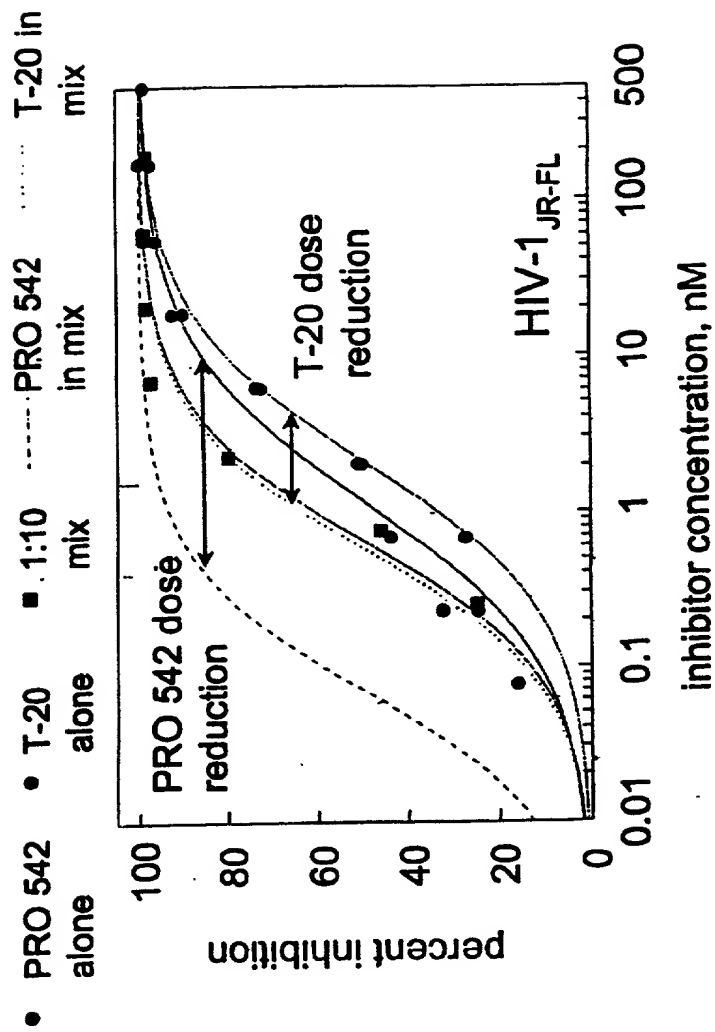


Figure 20

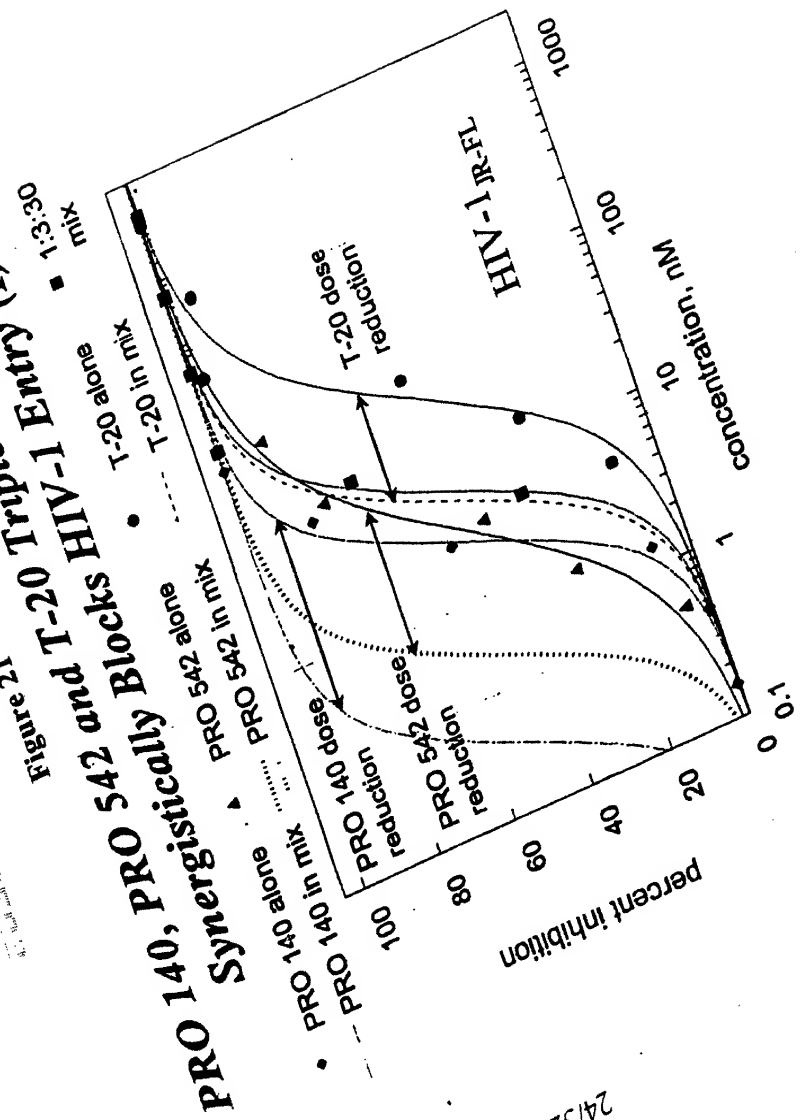
***Synergistic Inhibition of HIV-1 Cell-Cell Fusion
with PRO 542 and T-20 (II)***

Conc. Ratio	Percent Inhibition	Combination Index	Inhibitory Conc, nM		Dose Reduction (fold)	
			PRO 542	T-20	PRO 542	T-20
1:2	95	0.32	95	47	17	4.9
	90	0.38	39	22	13	4.2
	50	0.69	3.0	2.5	6.2	2.8
1:10	95	0.27	28	58	20	4.8
	90	0.28	11	22	20	4.5
	50	0.34	0.84	1.3	22	3.7
1:50	95	0.33	47	120	56	3.2
	90	0.34	15	42	50	3.2
	50	0.38	0.49	1.8	35	3.0

Virus: HIV-1_{JR-FL}

PRO 140, PRO 542 and T-20 Triple Combination

Figure 21
PRO 140, PRO 542 and T-20 Triple Combination



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Figure 22

**PRO 140, PRO 542, T-20 Triple Combination
Synergistically Blocks HIV-1 Entry (II)**

Percent Inhibition	Combination Index	Inhibitory Conc, nM			Dose Reduction (fold)		
		PRO 140	PRO 542	T-20	PRO 140	PRO 542	T-20
95	0.24	24	61	160	17	12	7.1
90	0.22	23	32	96	21	8.4	7.4
70	0.19	20	9.8	40	32	4.5	8.9
50	0.18	18	4.7	23	41	3.0	10

Inhibition of HIV-1_{JR-FL} mediated cell-cell fusion with PRO 140, PRO 542 and T-20 used in a 1:3:30 molar ratio.

Figure 23
PRO 542 Does Not Potentiate T-20 Activity in the Absence of Coreceptor

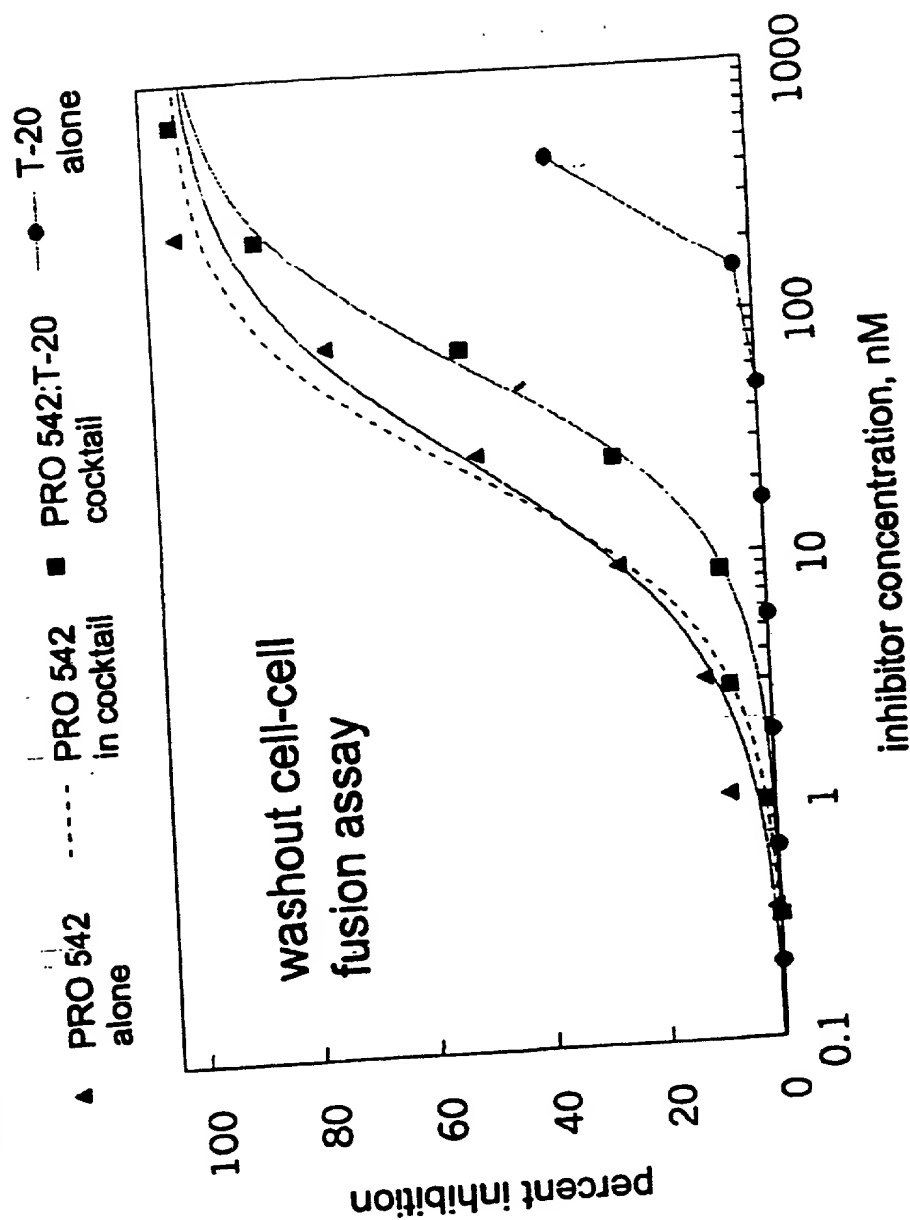


Figure 24

Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (I)

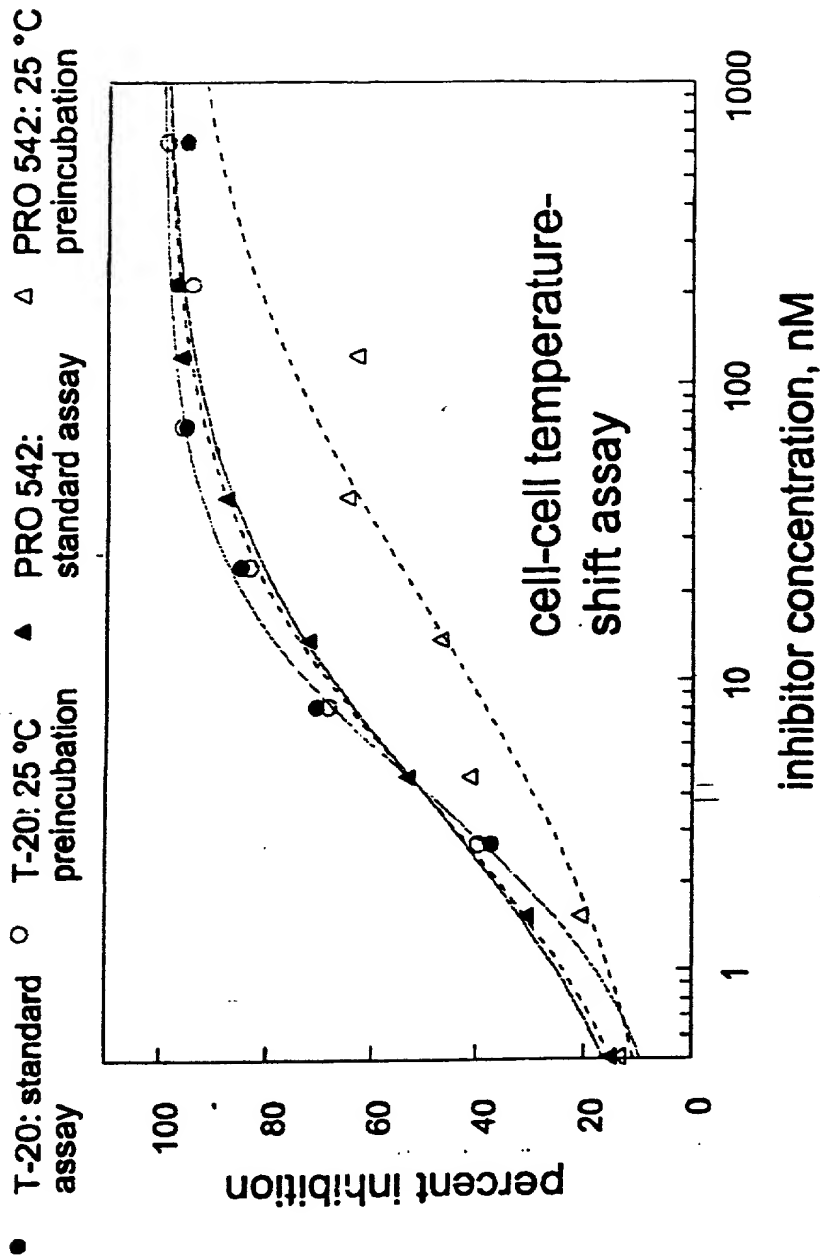


Figure 25

Formation of the Prehairpin Intermediate Requires CD4, Coreceptor and 37 °C (II)

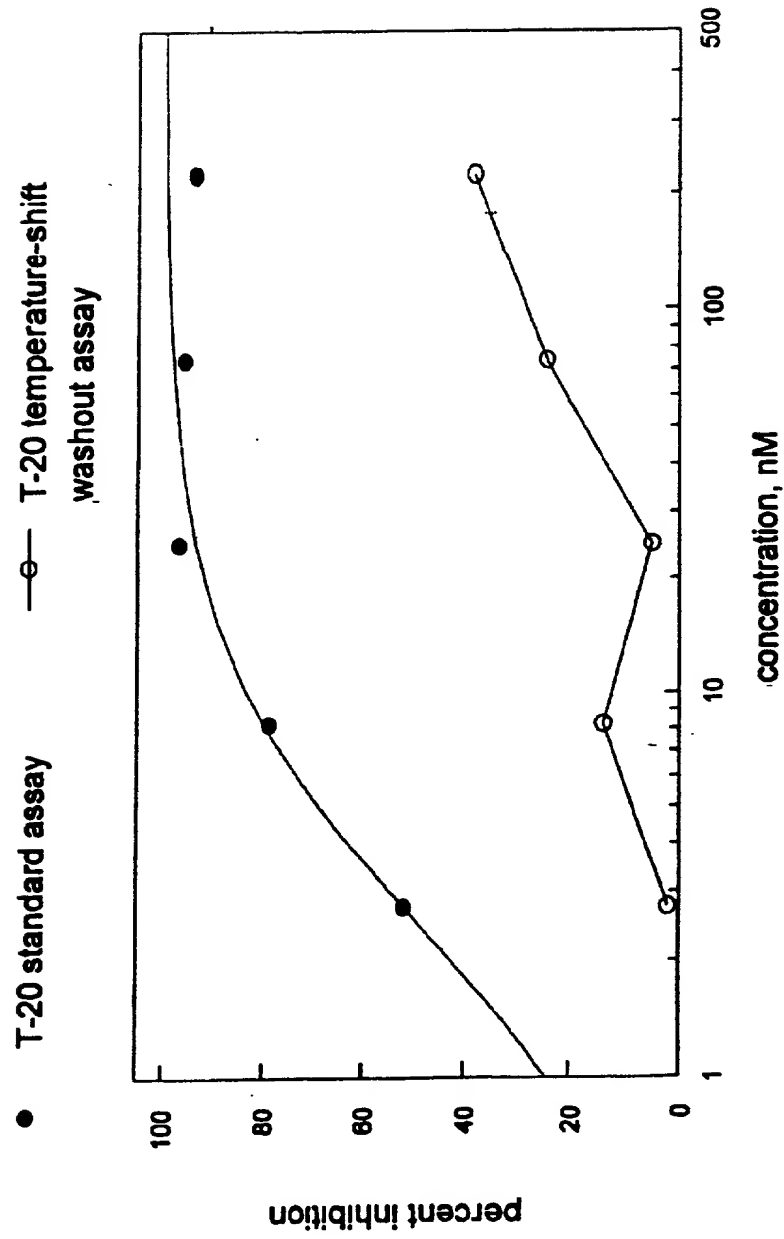


Figure 26

Possible Mechanism of Synergy: PRO 542 Increases the Half-Life of the T-20 Targets

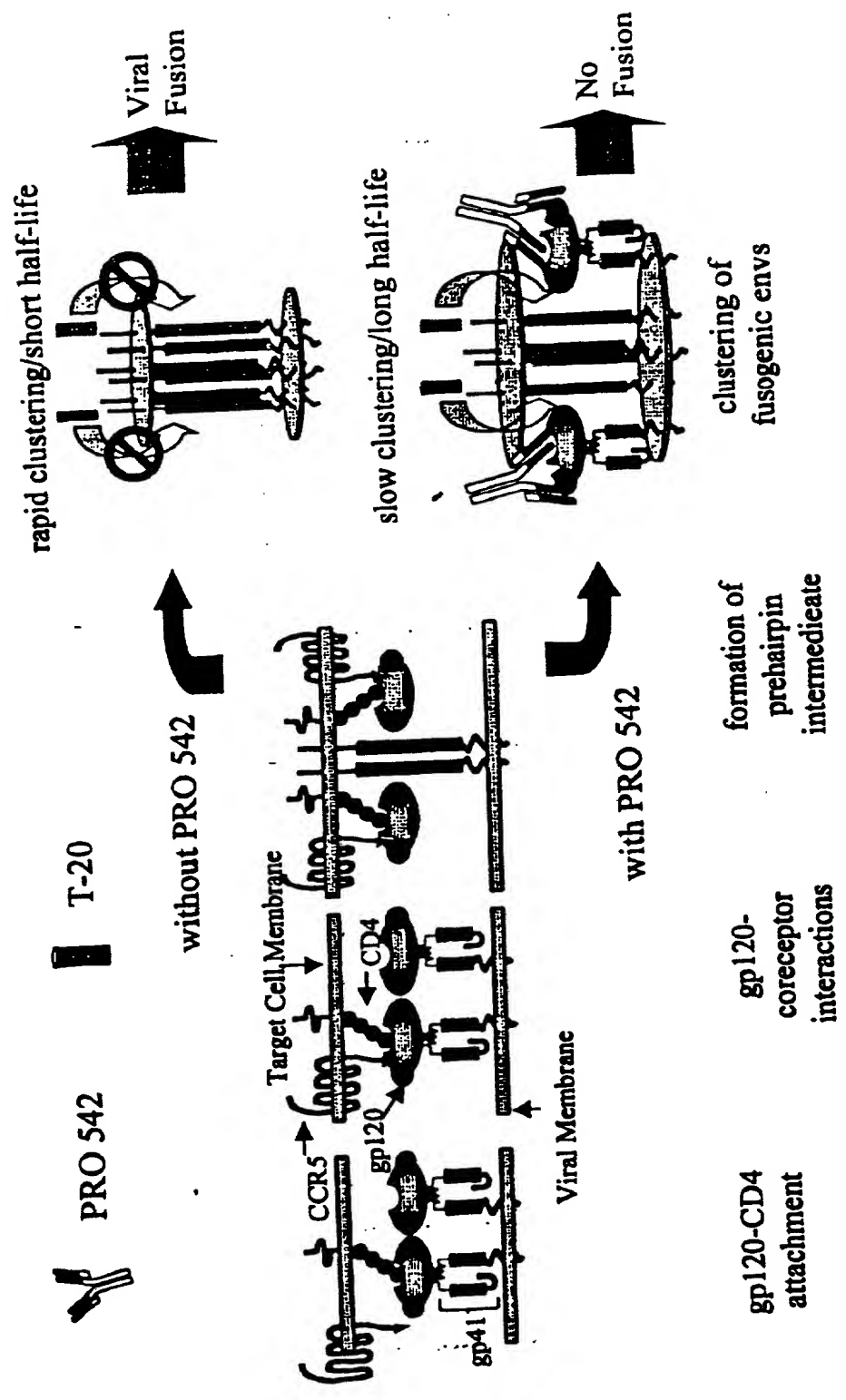


Figure 27

Possible Mechanism of Synergy: PRO 542 Increases the Half-Life of the T-20 Targets

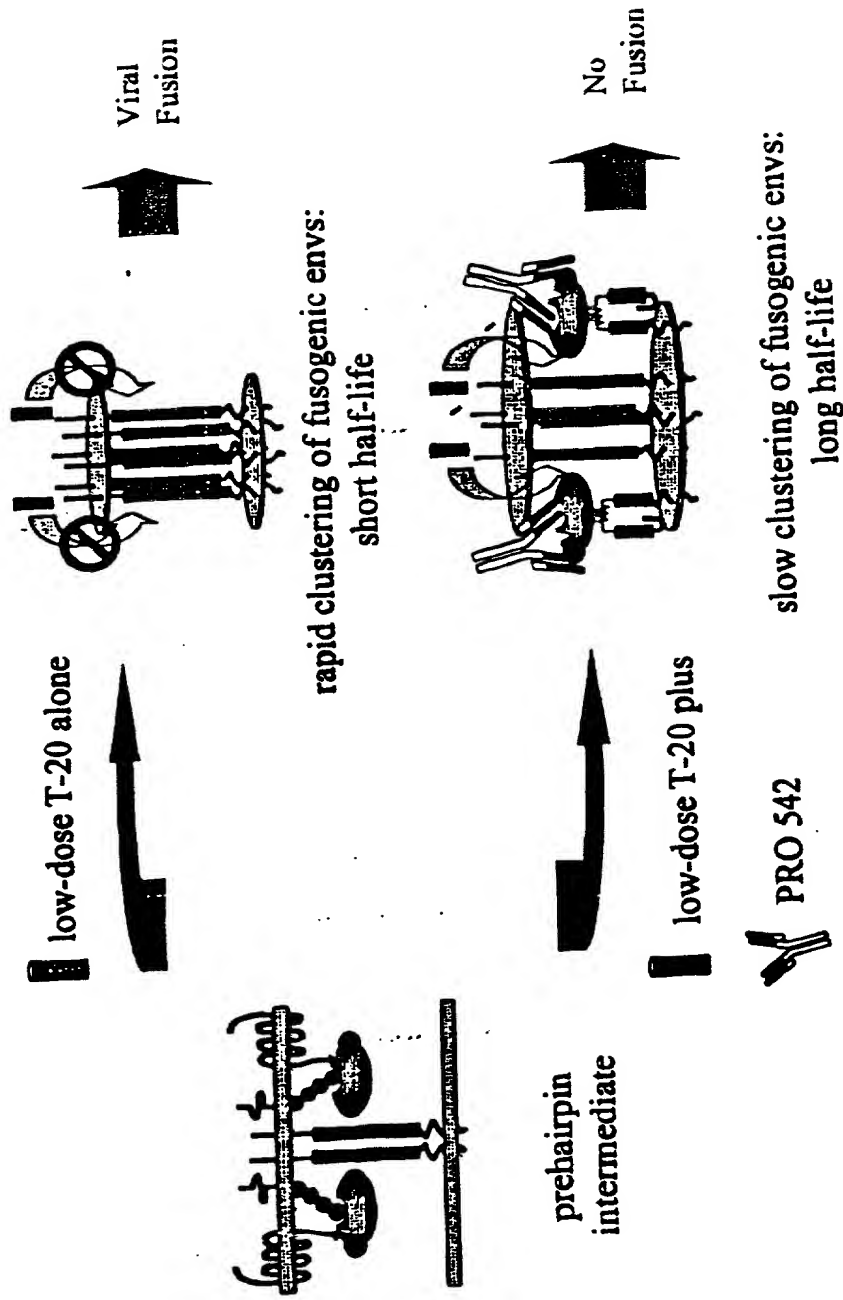


Figure 28

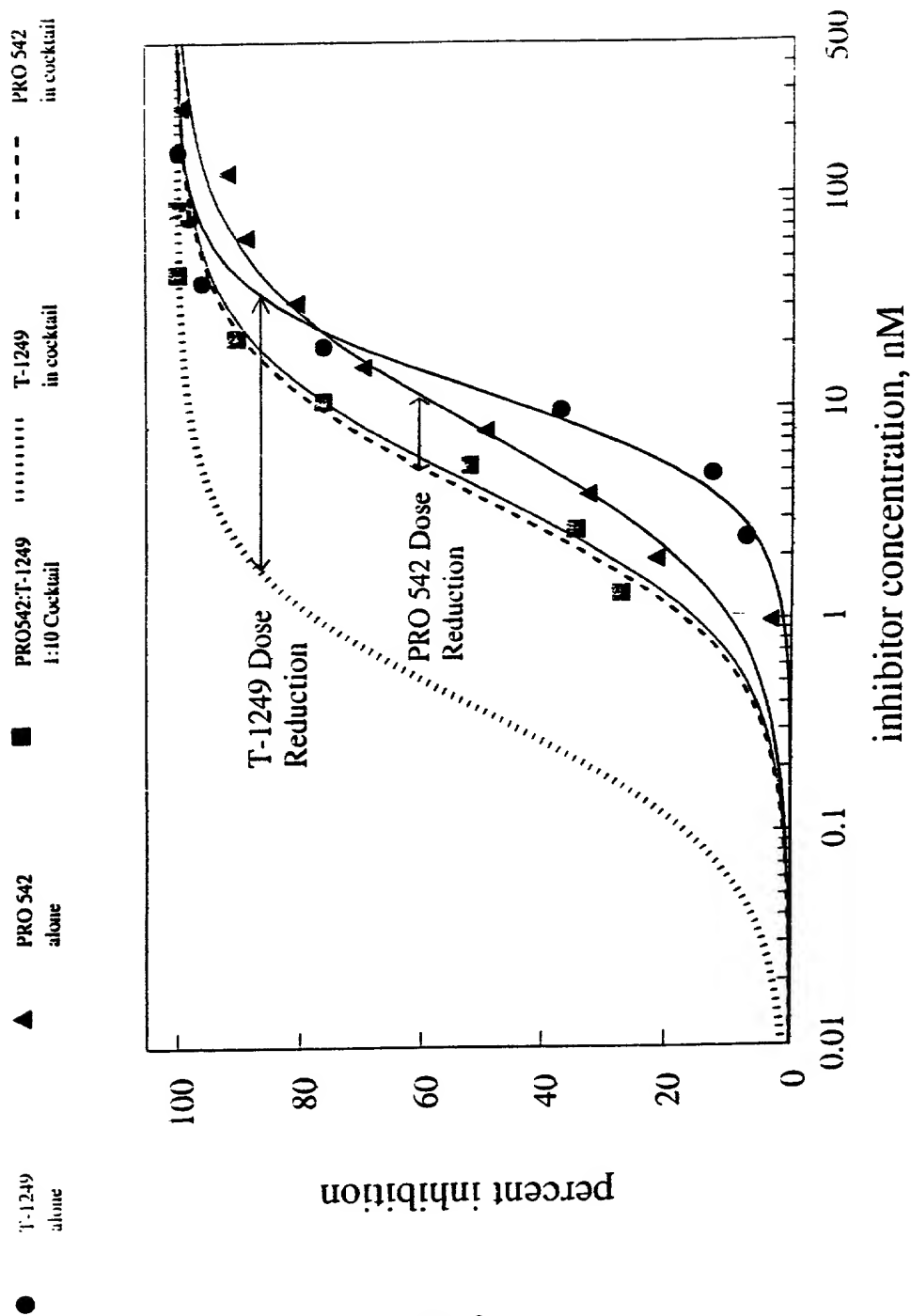


Figure 29

Fraction Inhibited	Dose PRO 542, nM (alone)		Dose PRO 542, nM (comb)		Dose T-1249, nM (alone)		Dose T-1249, nM (comb)		Combination Index		Dose Reduction PRO 542		Dose Reduction T-1249	
	nM (alone)	nM (comb)	nM (alone)	nM (comb)	nM (alone)	nM (comb)	nM (alone)	nM (comb)	Index		PRO 542		T-1249	
0.95	87.90	13.58	37.83	1.36	0.20	6.47	27.86							
0.90	48.69	9.52	27.11	0.95	0.24	5.12	28.48							
0.85	33.78	7.64	22.06	0.76	0.27	4.42	28.87							
0.80	25.65	6.47	18.88	0.65	0.30	3.96	29.17							
0.75	20.43	5.65	16.61	0.56	0.32	3.62	29.42							
0.70	16.75	5.01	14.85	0.50	0.34	3.34	29.64							
0.65	13.99	4.50	13.41	0.45	0.37	3.11	29.84							
0.60	11.81	4.06	12.20	0.41	0.39	2.91	30.03							
0.55	10.05	3.68	11.13	0.37	0.41	2.73	30.21							
0.50	8.57	3.35	10.18	0.33	0.44	2.56	30.39							